(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 1 236 474 A1

(12) EUROPEAN PATENT APPLICATION

(43) Date of publication:
04.09.2002 Bulletin 2002/36

(51) Int Cl.[7]: **A61K 38/17**, A61K 31/70, A61P 35/00

(21) Application number: **01104737.0**

(22) Date of filing: **26.02.2001**

(84) Designated Contracting States:
AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE TR
Designated Extension States:
AL LT LV MK RO SI

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(54) Negative regulation of epidermal growth factor receptor activity by Mig-6

(57) The present invention relates to the use of a Mig-6 protein or a nucleic acid coding therefor for the manufacture of an agent for the modulation of epidermal growth factor receptor (EGFR) activity. Further, the use of Mig-6 or nucleic acids coding therefor as a target for the modulation of EGFR receptor activity is disclosed. The invention also relates to a method for identifying novel substances capable of modulating EGFR activity. The modulation of EGFR activity preferably comprises an inhibition of the EGFR signal and is thus suitable for applications, particularly diagnostic or medical applications, wherein an inhibition of EGFR activity is desired. Thus, the present invention relates to novel methods for diagnosing, treating or preventing EGFR overexpression associated disorders such as tumors.

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induced endocytosis (Honegger et al., 1987; Lamaze and Schmid, 1995). After internalization, growth factor receptors get translocated into internal vesicles of the multivesicular body and segregated either for recycling or lysosomal/proteosomal degradation. This process of segregation is also involved in attenuation of RTK signalling and several molecules, such as annexin I (Futter et al., 1993), SNX1 (Kurten et al., 1996), PI3K (Joly et al., 1995); Grb2 (Wang and Moran, 1996) or c-Cbl (Levkowitz et al., 1998), have been suggested to be playing a role in it.

[0006] Desensitization of the EGFR may also involve the phosphorylation of receptor serine and threonine residues. Four major serine phosphorylation sites on the EGFR have been mapped; serine S671, S1002, S1046 and S1047 (Heisermann and Gill; 1988; Kuppuswamy et al., 1993). A role in the process of EGFR desensitization has been suggested for S1002 (Kuppuswamy et al., 1993) as well as for SS1046/1047 (Counterway et al., 1992; Theroux et al., 1992a; Theroux et al., 1992b). In addition, it has been shown that the protein serine/theronine kinase C (PKC) downmodulates the signalling potential of the EGFR by an MAPK/ERK kinase (MEK) dependent mechansim (Morrison et al., 1996) and that EGFR kinase activity is decreased by prolactin-induced threonine phosphorylation of the receptor (Quijano Jr. and Sheffield, 1998).

[0007] Besides the role of the EGF-signalling in normal mitogenesis, aberrant expression or structural alterations of the receptor or its ligand are involved in oncogenesis. The EGFR is amplified in human cancers includding bladder (Proctor et al., 1991), breast (Horak et al., 1991) and colon tumors (Radinsky et al., 1995) and different truncation mutants of the EGFR have been detected in glioblastoma and carcinomas of the lung, breast and ovary (Hesketh, 1997). In addition, the impairment of EGFR negative regulation has been connected to cell transformation and tumorigenicity (Wells et al., 1990; Ekstrand et al., 1992). In different *v*-erbB oncogenes, for example, the negative regulating serine phosphorylation sites SS1046/1047 are consistently found to be deleted and this mutation correlates with increased oncogenic potential (Theroux et al., 1992a). These examples indicate that comprehensive understanding of the mechanisms that negatively regulate mechanisms that negatively regulate RTK signalling will provide new insights into the process of oncogenesis and may yield novel strategies for cancer therapy.

[0008] In present study, we used a yeast two-hybrid approach to identify new interaction partners of the EGFR, which are involved in the negative regulation of its signal capacity. Among well known as well as unknown interaction partners, Mig-6 was identified. Mig-6 interacts with the EGFR in vivo in an EGF inducible manner. Overexpression in Cos-7 cells as well as in Rat-1 fibroblasts leads to accelerated dephosphorylation of the activated receptor. Mig-6 reduces MAPK activation upon EGF stimulation and suppresses focus formation induced by overexpression of the EGFR. Moreover, the transcription of Mig-6 mRNA is induced upon EGF stimulation which suggest a role of Mig-6 in negative feedback regulation of the EGFR signal.

[0009] A human Mig-6 gene was cloned by Wick et al. (1995). Recently Fiorentino et al. (2000) have described an inhibition of ErbB-2 mitogenic and transforming activity by RALT, a Mig-6 analog from rat, which binds to the ErbB-2 kinase domain. An interaction between Mig-6 and EGFR is, however, neither disclosed nor suggested.

[0010] Thus, a first aspect of the present invention relates to the use of a Mig-6 protein for the manufacture of an agent for the modulation, particularly for the inhibition of EGFR activity.

[0011] A further aspect of the present invention relates to the use of a nucleic acid encoding a Mig-6 protein or a nucleic acid complementary thereto for the manufacture of an agent for the modulation, particularly for the inhibition of EGFR activity.

[0012] A third aspect of the present invention relates to the use of a Mig-6 protein as a target for the modulation of EGFR activity.

[0013] A fourth aspect of the present invention relates to the use of a nucleic acid encoding a Mig-6 protein or a nucleic acid complementary thereto as a target for the modulation of EGFR activity.

[0014] A fifth aspect of the present invention relates to a method for identifying novel modulators of EGFR activity by screening for substances having an equivalent biological activity of Mig-6.

[0015] The term "Mig-6 protein" as used in the present application particularly encompasses mammalian Mig-6 proteins, such as Mig-6 proteins from man, mouse, rat, hamster, monkey, pig, etc. The sequence of several mammalian Mig-6 DNAs and the corresponding proteins are disclosed in Genbank Accession No. M23572 (rat), NM_018948 (human) and AK004851 (mouse) and citations therein, incorporated herein by reference.

[0016] Especially preferred is a human Mig-6 protein comprising:

(a) the amino acid sequence as shown in Genbank Accession No. NM_018948 or

(b) an amino acid sequence having an identity of at least 80%, particularly of at least 90% and more particularly of least 95% thereto, wherein the amino acid sequence identity may be determined by a suitable computer program such as GCG.

[0017] Furthermore, the term "Mig-6 protein" encompasses recombinant derivatives or variants thereof as well as fragments capable of binding to EGFR and preferably thereby inhibiting EGFR signal activity. Such derivatives, variants and fragments are obtainable by recombinant expression of corresponding nucleic acids in a suitable host cell and obtaining the resulting expression products by known methods. The activity of the re-

Mig-6 gene in this target cell.

[0027] Furthermore, an activator of Mig-6, e.g. a substance which enhances the biological activity of Mig-6, may be administered to the target cell.

[0028] Still a further embodiment of the present invention is a method of identifying novel modulators of EGFR activity comprising screening for substances having equivalent biological activity as Mig-6. In this context, the term "biological activity of Mig-6" preferably comprises binding to EGFR in a region between amino acids 985 and 995, wherein said binding is more preferably independent of tyrosine 992 and results in a selective inhibition of the mitogen-activated protein kinase in response to EGF.

[0029] The screening method may be a high-throughput screening method, wherein a plurality of substances is tested in parallel. The screening assay may be a cellular assay or a molecular assay, wherein an interaction of a substance to be tested with the Mig-6 binding region of EGFR is determined. The EGFR may be provided on a cell, preferably an EGFR overexpressing cell, an EGFR containing cell fraction or a substantially isolated and purified EGFR or a fragment thereof which is capable of binding Mig-6. Any active substance identified by this method may be used as a pharmaceutical agent or as a lead structure which is further modified to improve pharmaceutical properties.

[0030] The present invention is explained in more detail in the following figures and examples.

Figure description

[0031]

Fig. 1 A) Mig-6 homology comparison.
Mig-6, ACK1 and ACK2 contain a Cdc-42 and Rac interaction binding (CRIB) motif. Mig-6 and ACK1 share sequence homology within a carboxy-terminal serine- and proline-rich region, which is deleted in ACK2. In addition, ACK1 and ACK2 contain a kinase and an SH3 domain.
B) Amino acid homology of 71% in the serine- and proline-rich carboxy terminal domain between Mig-6 and ACK1 (shaded region in A). The amino acid sequences were aligned with the GCG computer program.

Fig. 2 Interaction between Mig-6 and the EGFR in the yeast two-hybrid system.

A) Representative examples of colonly growth. Laminin, the intracellular domain of the EGFR, the kinase impaired EGFR mutant K721A or the indicated truncations with full length Mig-6. The strains were streaked on synthetic media containing glucose or galactose and lacking leucine, tryptophan, histidine and uracil.

B) β-galactosidase activity assays. Strains containing the same constructs as in A) were grown in liquid synthetic media containing glucose or galactose and lacking tryptophan, histidine and uracil. o-Nitrophenyl β-D-galactopyranoside was used as a substrate and β-galactosidase activity was calculated from absorbance measurements (OD) at 420 nm and 600 nm and expressed as $OD_{420}/OD_{600}*1000$. The data from three independent experiments and the standard deviation are indicated.

Fig. 3 Co-immunoprecipitation of the endogenous EGFR with HA-Mig-6 and phosphoamino acid analysis of Mig-6.

A) Cos-7 cells were transiently transfected with cDNA encoding HA-Mig-6 or control-transfected with the empty vector. After starvation, the cells were stimulated with 10 ng EGF/ml for 10 min, lysed and HA-Mig-6 was immunoprecipitated with a monoclonal anti-HA antibody. After gel electrophoresis, the precipitated HA-Mig-6 was detected by immunoblotting with anti-HA antibody and the coprecipitated EGFR was detected with an anti-EGFR antibody. Expression of transfected HA-Mig-6 and endogenous EGFR was checked by probing the whole cell extract with the respective antibodies.
B) For phosphoamino acid analysis of Mig-6, Cos-7 cells were transfected with cDNA encoding Mig-6, starved and stimulated with 10 ng EGF/ml for different periods of time between 10 and 60 min (representive data of unstimulated and after 10 min stimulation are shown). After immunoprecipitation, the proteins were gel eletrophoresed, transferred to a PVDF membrane and subjected to acid hydrolysis. The amino acids were then separated by two-dimensional thin layer electrophoresis on cellulose plates and detected by autoradiography. Exposure was at -80°C using an intensifying screen.

Fig. 4 Mig-6 accelerates EGFR dephosphorylation and reduces MAPK activation upon EGF stimulation.

A) Cos-7 cells were transiently transfected with cDNA encoding Mig-6 or control-transfected with the empty vector. Quiescent cells were stimulated with 10 ng EGF/ml as indicated. After lysis the EGFR was precipitated with a monoclonal anti-EGFR and subsequently immunoblotted with a monoclonal anti-phosphotyrosine (α-PY) antibody. The amount of EGFR in immunoprecipitates was determined

HA epitope (Meloche et al., 1992). cDNAs used for viral infection were in pLXSN constructs. Mig-6 was cloned by PCR using the primers 5'-CGGAATTCGCCACCATGTCAACAGCAGGAGTTGCTGC-3' and 5'-CGCGGATCCTCAACACGCAGGAGTTGCTGCTC-3'. The PCR-product was digested with EcoR1 and Xbal and ligated into pcDNA3.1.

1.2 Yeast two hybrid screen

[0033] All protocols were performed essentially as described in the yeast protocols handbook (Clontech). A LexA-based, gal inducible yeast-two hybrid system was used (Gyuris et al., 1993). The yeast strain EGY 48 (MATa, trp1, ura3, his3, LEU2::pLEXAop6-LEU2), the plasmids pEG202, pSH18-34, pJG4-5 and a human liver library in pJG4-5 were gifts from Sugen Inc. A cDNA encoding human EGFR residues 645-1186 was subcloned into the LexA fusion expressing vector pEG202 by PCR. The yeast reporter strain EGY48 was sequentially transformed by the lithium-acetate method with the bait and library constructs. Positive interactor clones were selected by leucine auxotrophy and galactose-inducible β-galactosidase activity. The library construct was recovered from the positive clones with Escherichia coli strain KC8 (parF::Tn5, hsdr, leuB600, trpC9830, lacD74, strA, galK, hisB436) and retransformed into yeast strain EGY48 carrying a LexA-laminin fusion to eliminate unspecific positive clones. The sequence of the selected clones was used to search nucleotdide databases of the National Center for Biotechnology Information using the BLAST algorithm (Altschul et al., 1997).

1.3 Cell transfection, lysis, immunoprecipitation and immunoblotting

[0034] Cos-7 cells were transiently transfected using Lipofectamine essentially as described (Daub et al., 1997). 80-90% confluent cells were starved, stimulated as indicated and washed once with phosphate-buffered saline. Cells were lysed for 10 min on ice in buffer containing 50 mM Hepes pH 7.5, 150 mM NaCl, 1% Triton X-100, 1 mM EDTA, 10% glycerol, 10 mM sodium pyrophosphate, 2 mM sodium orthovanadate, 10 mM sodium fluoride, 1 mM phenylmethylsulfonyl fluoride and 10 µl/ml aprotinin. Lysates were pre-cleared by centrifugation at 13 000 r.p.m. for 10 min at 4°C. Supernatants were diluted with an equal volume of HNTG buffer (Seedorf et al., 1994) and subsequently immunoprecipitated using the respective antibodies and 30 µl of protein A-Sepharose for 4 h at 4°C. Precipitates were washed three times with HNTG buffer, suspended in SDS sample buffer, boiled and subjected to gel electrophoresis on 7.5% gels. Following SDS-PAGE, proteins were transferred to a nitrocellulose membrane and immunoblotted.

1.4 MAPK-assay

[0035] MAPK assays were performed as described previously (Daub et al., 1997). Epitope-tagged HA-Erk2 was immunoprecipitated from lysates obtained from 12-well dishes using 2.5 µg of 12CA5 antibody. Immunoprecipitates were washed three times with HNTG buffer and once with kinase buffer (20 mM Hepes pH 7.5, 10 mM $MgCl_2$, 1 mM dithiothreitol, 200µM sodium orthovanadate). Subsequently, kinase reactions were performed in 30 µl of kinase buffer supplemented with 0.5 mg/ml MBP, 50 µM ATP and 1 µCi of γ-^{32}P-ATP for 10 min at room temperature. Reactions were stopped by addition of 30 µl of 2 x SDS sample buffer and subjected to gel electrophoresis on 15% gels. The proteins were transferred to nitrocellulose and the upper part was probed with anti-Erk2 antibody. The labeled MBP in the lower part was quantified using a Phosphorimager (Fuji).

1.5 RNA extraction and Northern blot analysis

[0036] One 10 cm plate of 80-90% confluent Rat-1 cells was treated with inhibitors and agonists as indicated. Total RNA was isolated using the QIAshredder (QIAGEN) and RNeasy (QIAGEN) kits according to the manufacturers' recommendations. RNA samples (5-10 µg) were fractionated in a 1.4% agarose-formaldehyde gel and transferred to a nitrocellulose membrane by upward capillary transfer. cDNA probes were labeled with α-^{32}P-ATP using the megaprime labeling system from Amersham Pharmacia Biotech. Hybridization was carried out in formamide solution. Blots were exposed using intensifying screens.

1.6 Downregulation of the EGFR

[0037] Rat-1 cells stably expressing Mig-6 cDNA or control cells were superinfected with supernatants of GR+E 86 releasing high titer pLXSN-EGFR virus for 4-8 hours in the presence of Polybrene (4 µg/ml; Aldrich). After infection, cells were trypsinized and seeded on 12-well dishes (110'000 cells/well). 12 hours later cells were starved and stimulated with EGFR as indicated. The medium was aspirated and the cells were washed twice with ice-cold DHB (DMEM with 20 mM Hepes pH 7.5 and 0.1% bovine serum albumin (BSA)). Surface-bound ligand was removed by acid stripping at 0°C (200 mM acetic acid pH 2.5, 500 mM NaCl) for 6 min and cells were washed three times with DHB. The number of ligand-binding sites on the cell surface was then determined by incubating cells with ^{125}I-EGF at 4°C for at least 2 hours. After washing away the unbound radiolabeled ligand three times with ice-cold DHB the surface bound EGF was removed by acid stripping and the radioactivity was counted with a Beckmann 400 gamma counter.

from ^{32}P labeled cells further demonstrated the presence of phosphoserine and phosphothreonine but no phosphotyrosine in either the unstimulated state or upon EGF stimulation for up to 60 minutes. However, treatment of cells with the protein tyrosine phosphatase inhibitor pervanadate in Mig-6 tyrosine phosphorylation as shown by anti-phosphorylation as shown by anti-phosphotyrosine immunoblotting of the anti HA-tag immunoprecipitated HA-Mig-6 protein (data not shown). Taken together, these results allow the conclusion that Mig-6 is phosphorylated on serine and threonine residues and that the steady state level of tyrosine phosphorylation level of this protein is either undectably low i.e. tightly regulated by a PTP and/or it is substrate of a protein tyrosine kinase other than the EGFR.

[0045] The involvement of Mig-6 in EGFR-signalling was further investigated using Cos-7 cells transiently expressing Mig-6. First, we examined tyrosine phosphorylation of the EGFR in transfected and non transfected cells upon activation with EGF. After 2 min of stimulation, EGFR tyrosine phosphorylation was induced in both cell cultures, but no significant difference in phosphotyrosine content was detected. However, after a stimulation time of 10 min or longer, tyrosine phosphorylation of the EGFR was dramatically reduced in cells expressing Mig-6 relative to control cells (Figure 4A). These findings led us to question, if downstream signalling of the EGFR is also negatively influenced by expression of Mig-6. We therefore investigated the activation of mitogen-activated protein kinase (MAPK) Erk2 using a transiently expressed hemagglutinin (HA) epitope-tagged form of Erk2 (HA-Erk2) and measured the incorporation of ^{32}P in myelin basic protein (MBP). Expression of Mig-6 did reduce activation of Erk2 by 65% upon direct EGFR stimulation with EGF and by 50% and 62% upon EGFR transactivation by the GPCR agonists LPA or thrombin, respectively (Figure 4B). In contrast, Mig-6 had no influence on MAPK activation upon PDGF stimulation, whereas MAPK activity was even increased by 30% in Mig-6 expressing cells upon FGF stimulation (Figure 4B).

2.3 Mig-6 induced EGFR downregulation

[0046] The antagonistic effects of Mig-6 in EGFR-signalling could be explained by direct inhibition of EGFR kinase activity, by interference with the transphosphorylation reaction within the receptor dimer, by activation of an EGFR PTR or by an involvement of Mig-6 in the process of downregulation. Since internalization of RTKs upon stimulation is considered to be a major attenuation mechanism (Sorkin and Waters, 1993a), we analyzed the effect of Mig-6 on the fraction of surface-exposed EGF-binding sites in Rat-1 fibroblasts. Rat-1 cells stably expressing Mig-6 (clone 19 and 23) or control cells (mock) were generated by infection with a retrovirus containing the cDNA of Mig-6 or the empty vector as described in Materials and Methods. After stimulation, the cells were stripped to remove the surface-bound EGF and incubated with ^{125}I-EGF. After washing the surface bound radioactivity was counted. Within 5 min of EGF stimulation, only 25% of the surface EGF-binding sites disappeared on the control cells. In contrast, in cells that stably expressed Mig-6 about 45%-55% of the surface binding sites were downregulated within 5 min of stimulation (Figure 5). After 20 min of stimulation 20% of the surface receptors were still present on control cells compared to only 10% on the Mig-6 expressing cells. Interestingly, analysis of Mig-6 transfected and control cells indicated that the EGFR half life of the endogenous EGFR was extended by Mig-6 overexpression from 11 to 14 hours (data not shown) suggesting no involvement in EGFR degradation.

2.4 EGF induces Mig-6 mRNA expression

[0047] Since Mig-6 is involved in EGF-signal regulation and both human and rat cDNA were originally identified by screening libraries of stimulated cells (Chrapkiewicz et al., 1989; Lee et al., 1985; Wick et al., 1995), we investigated, if EGF induces Mig-6 mRNA production in Rat-1 fibroblasts. Northern blot analysis confirmed that Mig-6 mRNA is expressed only at low levels in starved cells (Figure 6A). Upon stimulation with EGF, Mig-6 mRNA levels are elevated within 30 min followed by a decline after one hour, whereas upon stimulation with FCS the highest expression is reached after one hour (Figure 6A). Similarly, the GPCR agonists LPA and thrombin, which transactivate the EGFR signal (Daub et al., 1996), also cause induction of Mig-6 mRNA expression (Figure 6B). To confirm that induction upon EGF, LPA, thrombin and FCS stimulation requires EGFR activity, we employed the EGFR-specific inhibitor AG1478. As shown in Figure 6B, pre-incubation of cells with AG1478 markedly reduced Mig-6 mRNA expression demonstrating the the EGFR signal was critical for Mig-6 gene transcription in response to EGF, FCS or the GPCR agonists LPA and thrombin. Stimulation with FGF did not induce expression of Mig-6 mRNA, whereas PDGF treatment induced a marginal effect (Figure 6C). To obtain information regarding the signalling pathways involved in Mig-6 gene activation we used pathway-selective inhibitors on EGF stimulated Rat-1 cells followed by RNA extraction and Northern blot analysis. No effect was seen with the p38 MAPK inhibitors SB203580 and SB202190 or with the PI3K inhibitors Ly29902 and wortmannin (data not shown). In contrast, the MAP kinase kinase (MEK) inhibitor PD98995 completely inhibited the induction of Mig-6 mRNA upon EGF stimulation (Figure 6D) demonstrating that activation of the ERK/MAPK pathway is necessary for Mig-6 transcription upon EGF stimulation.

2.5 Mig-6 inhibits EGF-induced cell transformation

[0048] EGFR gene amplification and overexpression

vation (Traverse et al., 1992). Mig-6 might represent an important element in a mechanism that defines the cellular response to EGFR ligand stimulation. A negative regulatory effect may either be due to enhanced dephosphorylation of the receptor or by its accelerated downregulation, a process that includes internalization and recycling or degradation. Since the overexpression of Mig-6 reduced the number of binding sites for EGF on the surface of cells, it seems likely that Mig-6 is involved in receptor downregulation. In this scenario Mig-6 could either recruit molecules involved in receptor endocytosis and therefore influence the kinetics of internalization or alternatively, the EGFR could be delivered more rapidly to a compartment where it is efficiently dephosphorylated and trapped in a "non-recycling" state after its internalization. Sorting of the receptor to the degradation pathway by Mig-6 can be excluded since expression of Mig-6 did not decrease the half life of the receptor. Since Mig-6 is involved in dephosphorylation as well as downregulation, the targeting of a phosphatase to the receptor could be an additional function of Mig-6. Furthermore, the small GTPase Rac has been described to regulate clathrin-coated vesicle formation and endocytosis. (Lamaze et al., 1996), which raises the interesting possibility of an interaction between Mig-6 and Rac and therefore an involvement of Rac in the downregulation of the EGFR.

[0054] Mig-6 does not only negatively regulate EGF-signalling, its transcription is also induced upon activation of the EGFR. In Rat-1 fibroblasts, Mig-6 mRNA expression is enhanced upon stimulation with FCS, EGF, LPA and thrombin, but not upon FGF and only marginaly upon PDGF stimulation. To induce Mig-6 transcription upon stimulation with FCS, LPA and thrombin the kinase activity is required. Thus Mig-6 appears to be part of a negative feedback mechanism that regulates the duration of the EGFR signal. Such negative feedback loops have been demonstrated in different major pathways, such as cytokine- (Endo et al., 1997; Naka et al., 1997; Starr et al., 1997), TGF-β- (Stroschein et al., 1999) or RTK-signalling (Seedorf et al., 1995; Ghiglione et al., 1999). In Drosophila oogenesis, the spatial activity of the EGFR is regulated by the two proteins kek1 and Argos in separate negative feedback loops. In this case the secreted protein Argos interacts in a paracrine manner directly with the extracellular domain of the EGFR (Freeman, 1996), whereas an interaction with the extracellular as well as the transmembrane domain of the EGFR is required for kek1 to inhibit the receptor (Ghiglione et al., 1999). In both cases the inhibitory proteins interact with the extracellular domain of the EGFR and might therefore inhibit the binding of the ligand to its receptor. Mig-6 on the contrary, is localized to the cytoplasm and interacts with the carboxy-terminal regulatory domain of the receptor which makes a direct interference with the binding of EGF unlikely. Whereas Argos and kek1 have only been described during Drosophila oogenesis, we found that Mig-6 is expressed in terminally differentiated tissues such as liver, kidney, lung and brain (data not shown). Thus, negative feedback regulation occurs not only during development and represents a critical mechanism for EGFR signal regulation and definition.

[0055] We have shown that enhanced expression of Mig-6 suppresses focus formation induced by EGFR overexpression but not by other oncogenes such as *v*-fms. This shows that Mig-6 specifically controls EGFR-signalling and impairment of this attenuation mechanism may lead to increased cell proliferation and tumor formation. Since the first demonstration of a connection between the EGFR and the *v*-erbB oncogene (Downward et al., 1984) and demonstration of EGFR gene amplification in A431 cells (Ullrich et al., 1984) the importance of this RTK as a major element in the promotion of tumor growth and invasion has been suggested for a variety of cancers. Bescause oncogenic dysregulation of RTK signals always involves the incapacitation of negatively regulating tumor suppressor gene products our findings regarding the downregulating function of Mig-6 provide new insights which have broad diagnostic and therapeutic significance.

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perproliferative disease.

12. The use of claim 10 or 11 wherein said disorder is selected from inflammatory processes and tumors.

13. Use of a Mig-6 protein as a target for the modulation of EGFR activity.

14. Use of a nucleic acid encoding a Mig-6 protein or a nucleic acid complementary thereto as a target for the modulation of EGFR activity.

15. The use of claim 13 or 14 comprising enhancing the amount and/or activity of a Mig-6 protein.

16. The use of claim 15 comprising an enhanced expression of Mig-6 in a target cell.

17. The use of claim 15 comprising the introduction of a Mig-6 protein activator to a target cell.

18. A composition comprising as an active agent a Mig-6 protein together with pharmaceutically acceptable carriers or diluents.

19. A composition comprising as an active agent a Mig-6 nucleic acid or a nucleic acid complementary thereto together with pharmaceutically acceptable carriers or diluents.

20. The composition of claim 18 or 19 for the use in diagnostic or medical applications.

21. The composition of any one of claims 18-20 for the use in tumor diagnosis, prevention or therapy.

22. A method of identifying novel modulators of EGFR activity comprising screening for substances having equivalent biological activity as Mig-6.

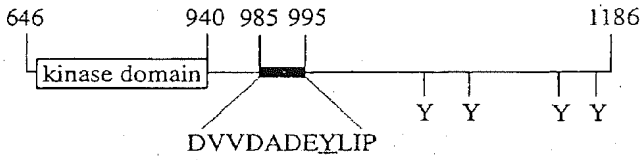
23. The method of claim 22 wherein said biological activity comprises binding to EGFR in a region between amino acids 985 and 995.

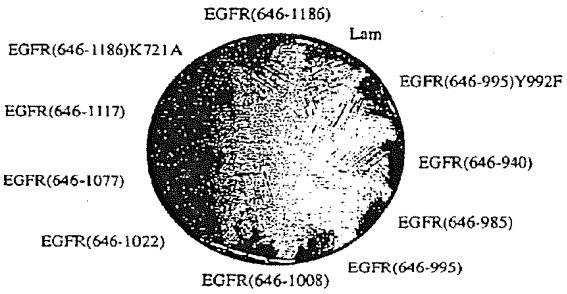
24. The method of claim 23 wherein said binding is independent of tyrosine 992.

25. The method of claim 22 wherein said biological activity comprises a selective inhibition of the mitogen-activated protein kinase pathway in response to EGF.

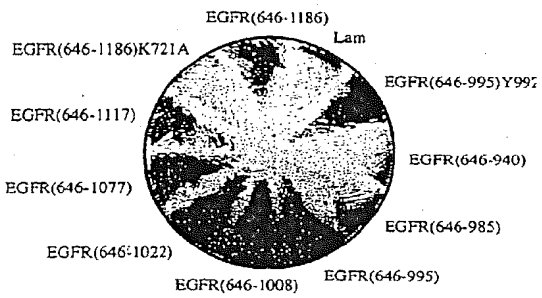
Fig. 2

A





Glucose



Galactose

B

LexA fusion	b-Galactosidase activity		Fold induction
	Glucose	Galactose	
Lam	8.0 +/- 2.3	8.8 +/- 0.7	1.1
EGFR(646-1186)	8.0 +/- 2.4	32.4 +/- 0.3	4.0
EGFR(646-1186)K721A	8.9 +/- 2.3	4.3 +/- 0.2	0.4
EGFR(646-1117)	27.2 +/- 2.1	186.3 +/- 32.2	6.8
EGFR(646-1077)	37.1 +/- 0.4	200.5 +/- 90.1	5.4
EGFR(646-1022)	19.5 +/- 2.7	111.7 +/- 10.3	5.7
EGFR(646-1008)	5.2 +/- 0.2	30.8 +/- 5.3	5.9
EGFR(646-995)	11.0 +/- 0.1	56.1 +/- 15.9	5.1
EGFR(646-995)Y992F	4.8 +/- 0.3	16.5 +/- 0.9	3.4
EGFR(646-985)	7.8 +/- 2.1	6.0 +/- 0.0	0.8
EGFR(646-940)	7.8 +/- 2.6	4.8 +/- 0.5	0.6

Fig. 4

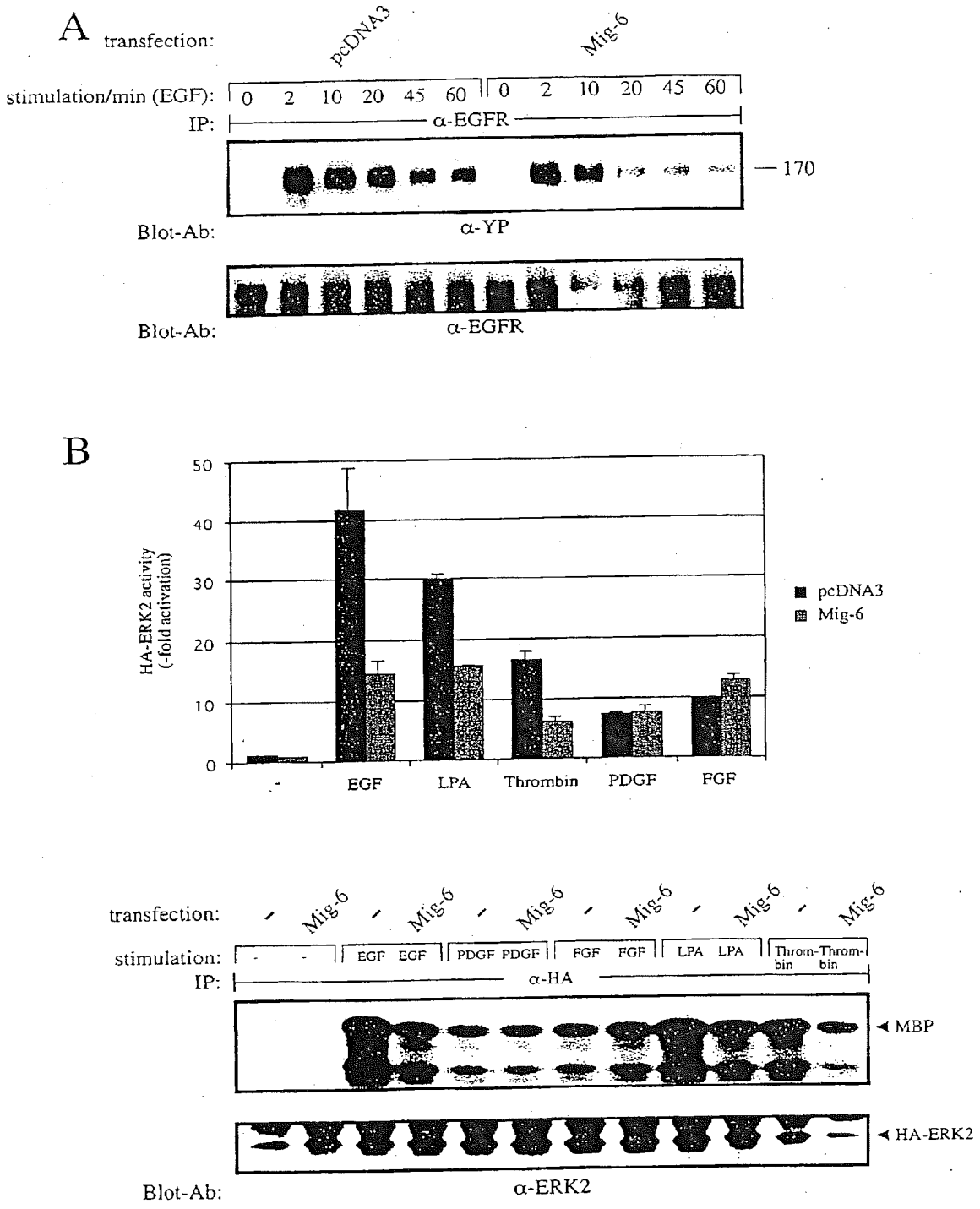
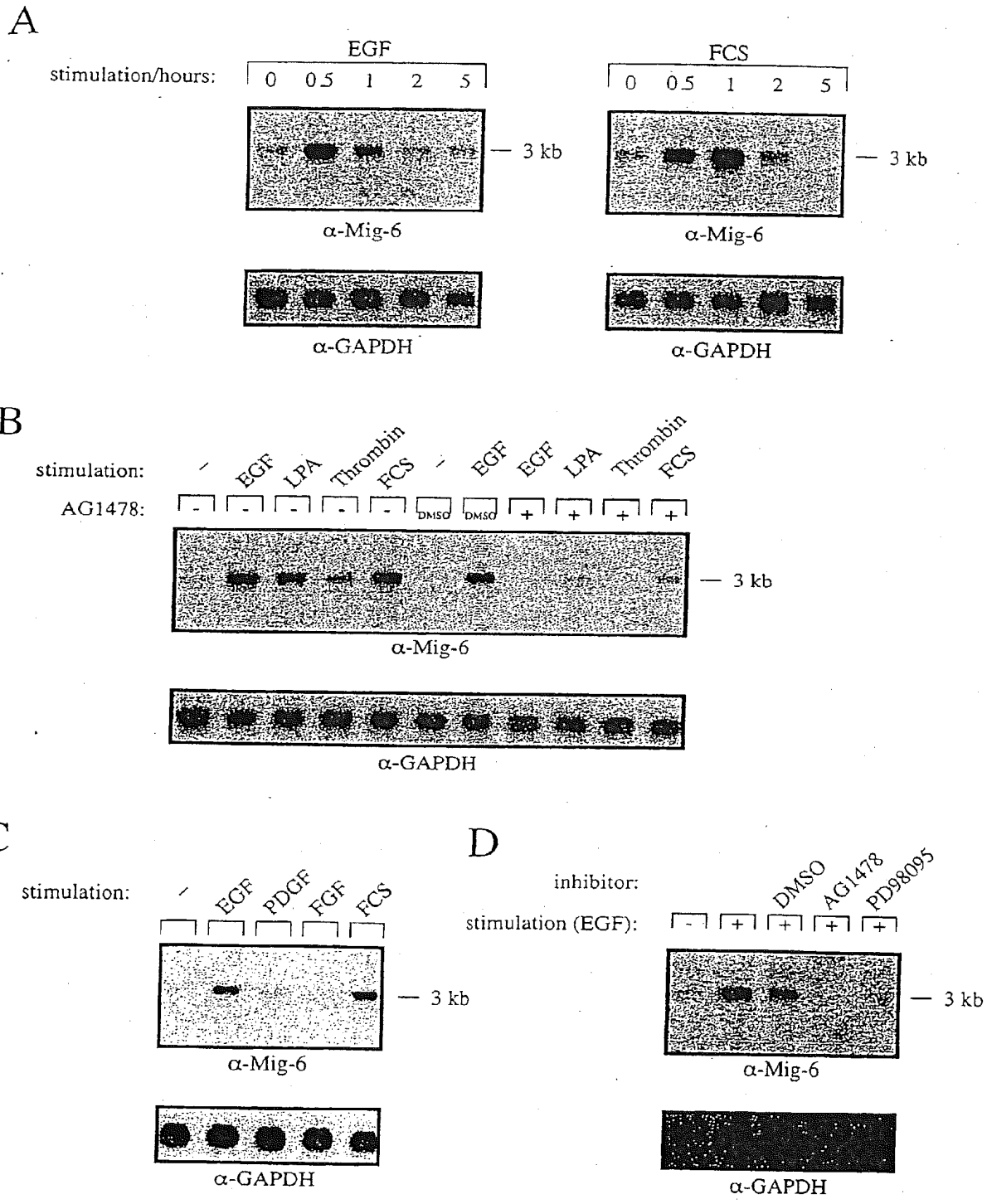


Fig. 6



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which under Rule 45 of the European Patent Convention shall be considered, for the purposes of subsequent proceedings, as the European search report

Application Number EP 01 10 4737

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
Y	FIORENTINO L ET AL: "INHIBITION OF ERBB-2 MITOGENIC AND TRANSFORMING ACTIVITY BY RALT, AMITOGEN-INDUCED SIGNAL TRANSDUCER WHICH BINDS TO THE ERBB-2 KINASE DOMAIN" MOLECULAR AND CELLULAR BIOLOGY, WASHINGTON, DC, US, vol. 20, no. 20, October 2000 (2000-10), pages 7735-7750, XP001008651 ISSN: 0270-7306 * see abstract, fig. 2, pages 7736-7737, 7741, 7747 and 7748 *	1-12, 18-25	A61K38/17 A61K31/70 A61P35/00
Y	CHEN W S ET AL: "FUNCTIONAL INDEPENDENCE OF THE EPIDERMAL GROWTH FACTOR RECEPTOR FROM A DOMAIN REQUIRED FOR LIGAND INDUCED INTERNALIZATION AND CALCIUM REGULATION" CELL, CELL PRESS, CAMBRIDGE, NA, US, vol. 59, no. 1, 6 October 1989 (1989-10-06), pages 33-43, XP001013054 ISSN: 0092-8674 * see abstract and fig. 7 *	1-12, 18-25	
	-/--		

TECHNICAL FIELDS SEARCHED (Int.Cl.7)

A61K
A61P

INCOMPLETE SEARCH

The Search Division considers that the present application, or one or more of its claims, does/do not comply with the EPC to such an extent that a meaningful search into the state of the art cannot be carried out, or can only be carried out partially, for these claims.

Claims searched completely :

1-12,18-25

Claims searched incompletely :

Claims not searched :

13-17

Reason for the limitation of the search:

The meaning and the category of claims 13-17 are so obscure that no meaningful search could be carried out

Place of search	Date of completion of the search	Examiner
MUNICH	4 September 2001	MERCKLING, V

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